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Site-Directed Cleavage of DNA by a Linker Histone−Fe(II) EDTA Conjugate: Localization of a Globular Domain Binding Site within a Nucleosome†

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ABSTRACT: The globular domain of linker histones specifically recognizes and binds to the nucleosome core. However, the exact location of the binding site of the globular domain has not been definitively elucidated. To address this issue, a linker histone has been specifically modified at a site adjacent to the globular domain with a radical-based DNA cleavage reagent. The linker histone−Fe(II) EDTA conjugate was bound to reconstituted nucleosomes containing a *Xenopus* 5S RNA gene, and the resulting cleavage of DNA was used to precisely map the location of the linker histone binding site. The results indicate that the binding site is located on the inside of the superhelical gyre of DNA, just inside the periphery of the nucleosome core region. The implications of these results for the binding of linker histones within native chromatin complexes are discussed.

Within the fundamental repeating unit of chromatin known as the nucleosome, about 160 bp of DNA is tightly wrapped in two superhelical turns around a central spool of protein comprised of two copies each of the four core histones (Kornberg, 1974; Kornberg & Thomas, 1974). In nearly all cases, a single molecule of linker histone (e.g., H1) also is associated with the core histone−DNA complex and the linker DNA between nucleosomes (Bates & Thomas, 1981; van Holde, 1989). The binding of linker histones serves to stabilize the 30 nm diameter chromatin fiber and perhaps higher order forms of chromatin (Widom, 1989; Shen et al., 1995), and modulation of linker histone binding activity is thought to be an important step in the potentiation/depotentiation of chromatin structure for transcription (Postnikov et al., 1991; Bresnick et al., 1992; Bouvet et al., 1994; Wolffe, 1995).

The association of linker histones with nucleosomes stabilizes an additional 20 base pairs of DNA immediately contiguous to the 146 base pair nucleosome core region[1] to digestion by micrococcal nuclease (Simpson, 1978). The appearance of the 168 bp chromatosome band has often been taken as diagnostic for the correct binding of linker histone to mono- or oligonucleosomal complexes (Allan et al., 1980; Buckle et al., 1992). The pioneering work of Allan et al. (1980) demonstrated that an 80 amino acid residue protease-resistant globular domain (Aviles et al., 1978) within linker histones specifically binds to the nucleosome core and protects DNA from micrococcal nuclease digestion like the full-length protein. Thus the small ca. 9 kDa structured globular domain provides for specific recognition of the 225 nucleosome core complex.

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[1] The nucleosome core region is defined as the 146 base pairs of nucleosomal DNA, most of which remains associated with the core histone octamer after micococcal nuclease digestion (van Holde, 1989). Core histone octamers associated with longer DNA fragments are referred to as nucleosome cores (distinct from core particles) to indicate the lack of linker histones within these complexes. Complexes which contain linker histones are referred to as nucleosomes. Chromatosomes are produced during micrococcal nuclease digestion and contain all four core histones, a single linker histone, and ∼168 base pairs of DNA.

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Considerable progress has been made in characterizing both the DNA and histone structures within the nucleosome (Richmond et al., 1984; Hayes et al., 1990; Arents et al., 1991; Arents & Moudrianakis, 1993). However, the exact nature and location of the specific binding site of the linker histone within the nucleosome have not been explicitly defined. On the basis of nuclease digestion experiments, it has been suggested that the globular domain is bound to the outward-facing minor groove of DNA at the center of the nucleosome, where the dyad axis of symmetry passes through the DNA (Allan, 1980). DNase I footprinting of linker histone-containing dinucleosome complexes supports this model (Staynov & Crane-Robinson, 1988). However, a comparison of the structure of the globular domain (Ramakrishnan et al., 1993) with other DNA binding proteins suggests that the globular domain associates with the major groove of DNA. Further, hydroxyl radical and DNase I footprinting of reconstituted complexes indicate that the minor groove of DNA at the dyad remains accessible to these reagents after linker histone association [Hayes & Wolffe, 1993; but see Staynov and Crane-Robinson (1988)]. Cross-linking between the globular domain of a linker histone and nucleosomal DNA occurs at a single site some distance away from the dyad axis of symmetry within a nucleosome (Hayes et al., 1994). These results have been interpreted as indicating that linker histone binds asymmetrically to the nucleosome, away from the dyad axis, perhaps on the inside of an outer superhelical gyre (Pruss et al., 1995). Consistent with this suggestion, neutron scattering experiments indicate that the mass of the linker histone is probably located closer to the center of mass of the nucleosome than would be allowed by specific association of the globular domain with the minor groove at the dyad (Baldwin, 1992).

Previous work has shown that a homogeneous population of nucleosome core complexes can be reconstituted *in vitro* from purified core histone proteins and a 238 base pair DNA fragment containing a *Xenopus borealis* 5S RNA gene (Rhodes, 1985). These complexes appear identical to native nucleosome complexes with regard to protein content, patterns of trypsin proteolysis sensitivity, comparisons of DNA structure by hydroxyl radical footprinting, and the protection of 146 base pairs of nucleosome core DNA from micrococcal nuclease digestion (Hayes et al., 1991; Hayes, unpublished). A majority of 5S nucleosome cores within a reconstituted sample exhibit identical translational and rotational positioning of the histone octamer with respect to the 5S sequence, thus making possible high-resolution footprinting studies of the structure of DNA in the nucleosome (Hayes et al., 1990). Moreover, linker histones bind to reconstituted 5S nucleosome cores in the correct stoichiometric ratio, and binding results in the production of chromatosome particles during micrococcal nuclease digestion, indicating that the native interaction between linker histone and the nucleosome core can be recapitulated *in vitro* with this system (Hayes & Wolffe, 1993). Interestingly, the affinity with which linker histones bind reconstituted 5S nucleosome core complexes is greater than that for naked 5S DNA, suggesting that assembly of the nucleosome complex results in a specific structure preferentially recognized by the linker histone (Hayes & Wolffe, 1993).

To address the issue of where the globular domain of linker histone recognizes and binds within the nucleosome core, I have employed a site-directed chemical mapping approach (Ermacora et al., 1992; Ebright et al., 1992; Lavoie et al., 1996; Heilek & Noller, 1996; Flaus et al., 1996). A linker histone protein was engineered so that a single residue at the edge of the globular domain was substituted for cysteine. A DNA cleavage reagent was covalently attached to the lone sulfhydryl group within the protein, and derivatized linker histones were incorporated into reconstituted 5S nucleosome complexes. The resulting localized DNA cleavage allowed a globular domain binding site to be clearly identified on the inside of the DNA superhelix, near the periphery of the nucleosome complex.

MATERIALS AND METHODS

DNA Fragments. A 238 bp *Hpa*II−*Dde*I fragment derived from plasmid pXbs-1 containing the *X. borealis* somatic 5S RNA gene was radiolabeled at the 5′ end of the noncoding strand at the *Hpa*II site 102 bp upstream from the initiation site for transcription of the 5S gene (+1) or on the coding strand at the *Dde*I site at position +137 as described (Hayes & Wolffe, 1993).

Preparation of H1° Mutant G101C. *Xenopus laevis* H1°a cDNA (Rutledge et al., 1988; Khochbin & Wolffe, 1993) was cloned by PCR methods from erythrocyte mRNA into the pET 3d vector (Novagen). The mutant G101C (hereafter referred to as H1°C) in which the codon for glycine at position 101 was changed to that for cysteine was prepared by standard PCR techniques in the same vector. Note that the globular domain in *Xenopus* H1°a, closely related to the avian H1 variant H5, extends from approximately amino acid residue 25 to 97 (Rutledge et al., 1988; Ramakrishnan et al., 1993). Proteins were expressed by treating BL21(DE3) cells containing these vectors and grown to early log phase with 0.4 mM IPTG[2] for 3 h at 37 °C. Cells were pelleted, treated with lysozyme, and sonicated, and insoluble components were separated by centrifugation at 5000*g*. Soluble proteins were fractionated by Bio-Rex (Bio-Rad, 50−100 mesh) cation-exchange chromatography, and H1°s eluted from the matrix with 20 mM Tris, pH 8.0, 2 mM EDTA, and 1.0 M NaCl. Fractions containing proteins were diluted 2-fold with 10 mM Tris-HCl, pH 8.0, and bound to a second Bio-Rex 70 column (100−200 mesh), eluted in the same way, and peak fractions (∼1 mg/mL) were stored in the elution buffer at −80 °C. Mutant and native H1° exhibited identical trypsin-resistant cleavage products (results not shown).

Modification of H1°C with EPD. H1°C (∼0.5 mg/mL in 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl) was fully reduced in the presence of 20 mM DTT for 2 h at 25 °C, DTT was removed by Bio-Rex 70 chromatography, and the reduced protein eluted in 10 mM Tris-HCl, pH 8.0, and 1 M NaCl. Fractions containing reduced protein (∼0.5 mg/mL) were quickly frozen on dry ice or reacted with a 1.1 equiv of Fe(II) EDTA-2-aminoethyl 2-pyridyl disulfide (EPD) (kindly provided by Drs. Robert Fox and David Ledman, University of Texas, Galveston) for 1 h in the dark at room temperature (Ermacora et al., 1992; Ebright et al., 1992). Disulfide exchange between the EPD and H1°C resulted in attachment of a Fe(II) EDTA complex via a three-atom tether to the

[2] Abbreviations: EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; IPTG, isopropyl β-D-thiogalactopyranoside.

lone cysteine residue at position 101 within the protein. The degree of modification was monitored by subsequent reaction of a portion of the modification reaction with a 2-fold molar excess [^{14}C]-*N*-ethylmaleimide (NEM) to label any remaining free sulfhydryls. The NEM labeling reaction was quenched by addition of DTT to 50 mM concentration and the sample applied to a protein gel. After being stained for proteins, the gels were dried and exposed to Kodak MR 5 autoradiographic film.

Reconstitution of Nucleosome Cores. Nucleosome core particles were prepared as described (Wolffe & Hayes, 1993). Nucleosome cores were reconstituted onto radiolabeled 5S DNA fragments by exchange with core particles as described except that EDTA was omitted from all buffers (Tatchell & van Holde, 1977; Ura et al., 1994). A typical 20 μL exchange reaction contained 1 M NaCl, 1.5 μg of donor core particles, 0.3 μg of naked nonspecific DNA, and 50−100 ng of labeled 5S fragment. After exchange and dilution about 50−60% of the labeled 5S fragments were assembled into mononucleosome cores without detectable dinucleosome complexes as monitored by nucleoprotein gel electrophoresis (Wolffe & Hayes, 1993).

Cleavage of DNA within H1°C−Fe(II) EDTA-Bound Nucleosomes. Approximately 400 ng of reconstituted nucleosome cores were incubated with 50 ng of unmodified H1°C or H1°C−Fe(II) EDTA in binding buffer (10 mM Tris-HCl, pH 8.0/50 mM NaCl/10 μM EDTA) for 15−20 min in the dark. Localized cleavage of the DNA was initiated by the addition of sodium ascorbate and hydrogen peroxide to a final concentration of 1 mM and 0.0075%, respectively, and allowed to proceed for 10−30 min in the dark at room temperature, as indicated in the figure legends. The reduction of hydrogen peroxide by the Fe(II) results in the localized production of hydroxyl radicals. Reactions were quenched by the addition of $^1/_{10}$th volume of 50% glycerol/10 mM EDTA, and the samples were immediately loaded onto preparative nucleoprotein gels as described (Wolffe & Hayes, 1993). After electrophoresis, the wet gels were autoradiographed, the linker histone-bound complexes were excised, and the labeled DNA was extracted and analyzed by sequencing gel electrophoresis. Hydroxyl radical footprinting of nucleosomal DNA was carried out as described (Wolffe & Hayes, 1993).

Linker Histone Gel Shift Assay. Samples containing approximately 25 ng of nucleosome core particles and ≤1 nM reconstituted 5S nucleosome were incubated with various amounts of H1°C (see figure legends) in 10 μL of binding buffer [10 mM Tris-HCl, pH 8.0/50 mM NaCl/1mM EDTA/5% (v/v) glycerol]. Samples were incubated at room temperature for 15−30 min and loaded directly onto running 0.7% agarose gels in 0.5× TBE (1× is 90 mM Tris base/90 mM boric acid/2.5 mM EDTA). Control experiments in which native linker histones were exchanged onto 5S nucleosome cores from chicken erythrocyte chromatin in the same buffer showed identical shifted bands (results not shown). After electrophoresis, the gels were dried and autoradiographed.

RESULTS

A linker histone protein was generated to facilitate site-specific attachment of a DNA cleavage reagent. The coding sequence for a somatic linker histone variant, H1°a, from *Xenopus* (Rutledge et al., 1988) was altered so that a single amino acid residue immediately adjacent to the carboxyl-terminal side of the globular domain was substituted for cysteine (Figure 1A). This position was chosen because of its proximity to the globular domain and because it was reasoned that substitution of an uncharged residue in the flexible C-terminal tail would be less detrimental to the binding activity of the protein than altering the sequence of the structured globular domain. The mutated protein, H1°C, was expressed in a bacterial system, purified to homogeneity, and modified with the cysteine-specific reagent EPD by disulfide exchange (Figures 1B and 2A) (Ermacora et al., 1992; Ebright et al., 1992; see above). This modification results in the attachment of a redox-active Fe(II) EDTA complex connected by a short three-atom tether to the sulfur of the lone cysteine residue within the protein to produce H1°C−Fe (II) EDTA. Subsequent modification with [^{14}C]-NEM showed that ≥95% of the protein was modified with EPD in this manner (Figure 2A, cf. lanes 2 and 3). A control shows that significant interprotein disulfide cross-linking does not occur during the incubation (Figure 2A, lanes 1 and 2).

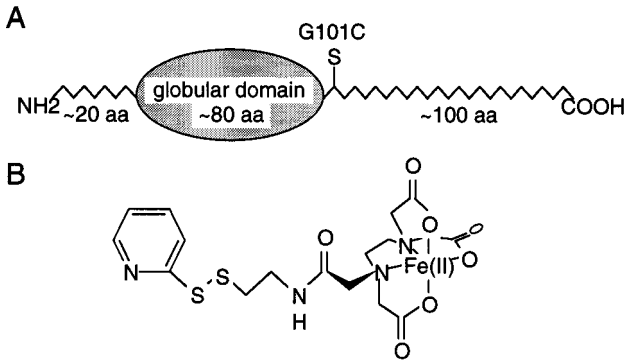

FIGURE 1: (A) Schematic of H1°C showing the position of glycine substituted for cysteine. The flexible N- and C-terminal tail domains are indicated flanking the central globular domain. (B) Structure of EPD. After disulfide exchange, the maximum distance between the Cα carbon atom of the modified cysteine and the metal center is ~14 Å (Lavoie et al., 1996).

Recombinant *Xenopus* H1°a binds the nucleosome in the same manner and with approximately the same affinity as native H1s isolated from calf thymus or H5 from chicken erythrocyte chromatin (J. J. Hayes, unpublished). Further, substitution of glycine for cysteine at position 101 in H1° and modification of the sulfhydryl with EPD have little effect on the affinity of the protein for binding to the nucleosome (Figure 2B) or on the ability of the protein to cause production of chromatosomes during a micrococcal nuclease digestion (Figure 2C). Importantly, the EPD-modified protein still binds preferentially to the reconstituted nucleosome core over the naked DNA (Figure 2B) (Hayes & Wolffe, 1993).

For the first experiments, nucleosome cores were reconstituted with 5S DNA radiolabeled on the 5′ end of the coding strand at the *Dde*I site located downstream of the 5S RNA gene at position +137 (see Figure 3). Reconstituted nucleosomes were mixed with a stoichiometric quantity of H1°C−Fe(II) EDTA and site-specific hydroxyl radical-mediated cleavage of the DNA in these complexes was carried out as described. Cleaved complexes were separated on preparative nucleoprotein gels, the band corresponding to the H1°−nucleosome complex was cut out, and the labeled DNA was recovered and analyzed on sequencing gels.

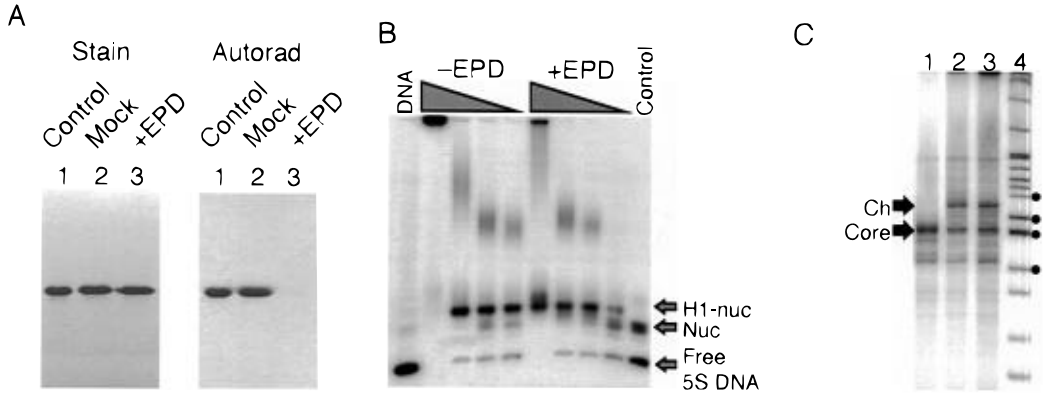

FIGURE 2: Modification of H1°C with EPD and nucleosome binding activity of modified protein. (A) H1°C is quantitatively modified with EPD. H1°C was incubated in the absence or presence of a 1.1-fold excess of EPD and then postmodified with [^{14}C]NEM to detect unreacted cysteine sulfhydryls. The samples were separated by SDS−PAGE, the gel was stained and photographed to locate proteins, and then labeled proteins were detected by autoradiography, as indicated. Lanes: 1, H1°C control reacted directly with [^{14}C]NEM; 2 and 3, H1°C mock treated or incubated with EPD, respectively. (B) Modification of H1°C with EPD does not alter preferential nucleosome binding activity. Increasing quantities (1.25, 2.5, 5, and 10 ng), as indicated, of H1°C or the H1°C−Fe(II) EDTA complex were incubated with 25 ng of nucleosome cores reconstituted with radioactively end-labeled 5S DNA. Samples were analyzed on agarose nucleoprotein gels and the gels autoradiographed. The positions of free DNA, nucleosome (Nuc), and H1°−nucleosome complexes (H1−Nuc) are indicated. Naked DNA and 5S nucleosome cores only were run in lanes DNA and control, respectively. (C) H1°C−Fe(II) EDTA efficiently promotes production of chromatosomes during a micrococcal nuclease digestion. Nucleosome cores (40 ng), reconstituted with internally labeled 5S DNA (Ura et al., 1994), were incubated with buffer and 5 ng of H1°C or H1°C−Fe(II) EDTA (lanes 1−3, respectively), and the complexes were digested with 0.6 unit of micrococcal nuclease and the products of digestion analyzed as described (Hayes & Wolffe, 1993). An autoradiograph of the nondenaturing gel is shown. Lane 4 contains labeled fragments of a *Msp*I digestion of pBR322 with fragments of 123, 147, 160, and 180 base pairs highlighted by dots. The positions of digestion products corresponding to the nucleosome core DNA (146 bp) and chromatosome length DNA (168 bp) are indicated.

Because of the high background (i.e., non-H1°C−Fe(II) EDTA-specific) cleavage associated with this experiment, it was necessary to compare the results to control reactions in which nucleosomes were incubated with H1°C unconjugated to Fe(II) EDTA and digested in the same way (Figure 3, lane 3).

Inspection of the cleavage pattern in DNA from H1°C−Fe(II) EDTA-bound complexes reveals a strong group of H1°C−Fe(II) EDTA-dependent cleavages centered at positions +62/+63 and a weaker but reproducible group of cleavages centered at +73 (Figure 3, lanes 4 and 5). All other cleavages observed in the resolved portion of the gel were also found in the control reaction and were thus not H1°C−Fe(II) EDTA-dependent (Figure 3, compare lane 3 with lanes 4 and 5). For reference, the hydroxyl radical footprint of the 5S nucleosome core reveals precisely where the DNA backbone is oriented away from the histone surface (Figure 3, lane 2 and black arrows) (Hayes et al., 1990). The two groups of H1°C−Fe(II) EDTA-dependent cleavages are spaced 10−11 base pairs apart, similar to the repeat of the DNA helix, and appear out of register with the peaks of cleavage found in the hydroxyl radical footprint of the nucleosomal DNA (cf. lanes 2 and 5). Thus the H1°C−Fe(II) EDTA-specific cleavage sites are located on the inside of the superhelical turn of DNA within the nucleosome, near to where the core histones contact the nucleosomal DNA. Comparisons of the region of DNA in contact with the core histone proteins reveal that the H1°C−Fe(II) EDTA-dependent cleavages map to near the edge of the nucleosome core region. No obvious H1°C−Fe(II) EDTA-specific cleavages are observed in the vicinity of the dyad axis of symmetry (Figure 3, open arrow; see Discussion).

To locate possible H1°C−Fe(II) EDTA-specific cleavages on the opposite end of the nucleosome complex (i.e., the region represented in the unresolved top portion of the gel in Figure 3), 5S DNA was 5′ end-labeled on the noncoding strand at the *Hpa*II site upstream of the 5S gene (Figure 4) and the experiment was repeated. No obvious H1°C−Fe(II) EDTA-dependent cleavages were observed in the "upstream" portion of the 5S nucleosome (Figure 4, lower part of the gel). However, three groups of cleavages centered at positions +57, +65, and +75 within 5S DNA were observed near the top of the gel (Figure 4, lane 3; gray arrows). Other bands found on this lane were also observed in the control and thus were not H1°C−Fe(II) EDTA-dependent (Figure 4, cf. lanes 2 and 3). Densitometric analysis revealed that the relative amount of specific cleavage at positions +57, +65, and +75 is approximately 0.75:1.0:0.75, respectively. These cleavages occur at the same location but on the complementary strand as those found in Figure 3. A comparison reveals that these H1°C−Fe(II) EDTA-dependent cleavages also are out of register with the hydroxyl radical footprint of 5S DNA in the nucleosome. As above, this indicates that the H1°C−Fe(II) EDTA-specific cleavage sites are located on the inside of the superhelical turn of DNA within the nucleosome, near to where the core histones contact the nucleosomal DNA.

DISCUSSION

The association of linker histones in chromatin is a fundamental step in the formation of the mature chromatin fiber. The protease-sensitive N- and C-terminal tail domains of linker histones are highly basic and probably bind and partially neutralize the polyanionic charge of the linker DNA between nucleosomes in the condensed chromatin fiber (Allan, 1980; Clark & Kimura, 1990). The protease-resistant globular domain of linker histones recognizes and binds the specific structure of the nucleosome (Allan et al., 1980). However, the location of the binding site of the globular domain on the surface of the nucleosome core has not been rigorously defined.

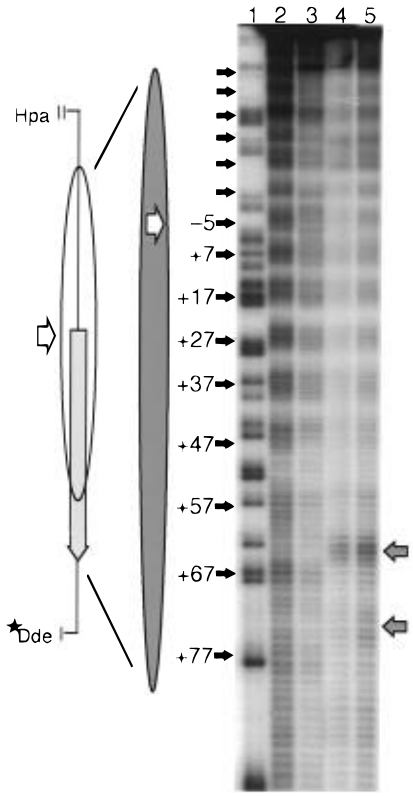

FIGURE 3: H1°C−Fe(II) EDTA-specific cleavages on the coding strand of 5S nucleosomal DNA. Nucleosomes were reconstituted with 5S DNA radioactively end-labeled at the *Dde*I site located downstream of the 5S nucleosome core region (see schematic). Nucleosomes were incubated with buffer and H1°C or H1°C−Fe-(II) EDTA, cleavage was initiated, and samples were prepared as described. Lanes 1: G-specific cleavage reaction markers; 2, hydroxyl radical footprint of the coding strand of 5S nucleosomal DNA; 3, control DNA from 5S nucleosomes bound by H1°C and incubated with cleavage reagents for 30 min; 4 and 5, DNA from nucleosomes bound by H1°C−Fe(II) EDTA and incubated with cleavage reagents for 10 or 30 min, respectively. H1°C−Fe(II) EDTA-specific cleavages are highlighted by the gray arrows; the positions of peaks in the hydroxyl radical footprint are indicated by the black arrows. The schematic (left) indicates the orientation of the 5S DNA fragment (vertical line) with respect to the gel and the position of the radioactive label (star) with regard to the 5S gene coding sequence (vertical thick arrow). The region of 5S DNA which contacts core histone proteins and the location of nucleosomal DNA on the gel are indicated by the open and gray ovals, respectively. The position of the nucleosomal dyad is indicated by the open arrow in both representations.

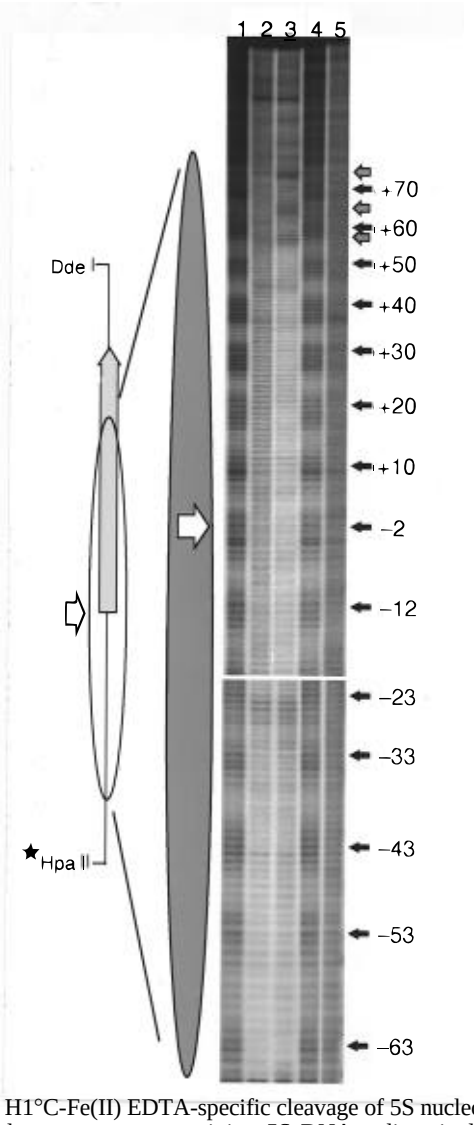

FIGURE 4: H1°C-Fe(II) EDTA-specific cleavage of 5S nucleosomal DNA. Nucleosome cores containing 5S DNA radioactively end-labeled on the noncoding strand at the *Hpa*II site located at position −102 (see schematic) were complexed with either unmodified H1°C or H1°C−Fe(II) EDTA. Complexes were incubated with cleavage reagents for 30 min and DNA cleavages analyzed by autoradiography of sequencing gels. DNA from nucleosomes incubated with unmodified H1°C as a control or H1°C−Fe(II) EDTA is shown in lanes 2 and 3, respectively. Lanes 1 and 4 show the hydroxyl radical footprint of the 5S nucleosome, and lane 5 shows the hydroxyl radical cleavage pattern of naked DNA. H1°C−Fe(II) EDTA-specific cleavages and the positions of peaks in the hydroxyl radical footprint are indicated as in Figure 3. The orientation of the 5S DNA fragment with respect to the gel, the position of the radioactive label, and the location of nucleosomal DNA on the gel are indicated as in Figure 3. The samples were separated on two different gels run for longer and short times in the upper and lower halves of the figure, respectively.

Several models for the location of the globular domain binding site on the nucleosome core are shown in Figure 5. A model based on nuclease digestions of linker histone-containing bulk chromatin complexes suggests that the globular domain binds to the outside of the superhelix of DNA at the center of the nucleosome and makes symmetrical interactions with the nucleosomal DNA (Allan et al., 1980; Staynov & Crane-Robinson, 1988) (Figure 5A). A second model (Figure 5B), derived from neutron scattering results (Lambert et al., 1991), suggests that the globular domain is located closer to the center of mass of the complex, perhaps between the superhelical gyres of DNA away from the dyad axis of symmetry (Baldwin, 1992). A third model (Figure 5C) is supported by protein−DNA cross-linking experiments, nuclease and hydroxyl radical footprinting of linker histone-containing complexes, and an analysis of the protein structure of the nucleosome core (Hayes et al., 1994; Pruss et al., 1995). This model suggests that the globular domain binds on the inside of the outer superhelical gyre of DNA, about 65 base pairs from the dyad axis [position +6.5 as defined in Richmond et al. (1984)], and implies that allosteric changes in the histone protein conformation upon H1 binding contribute to the observed chromatosome stop (Pruss et al., 1995).

I have determined the location of the globular domain binding site within a model nucleosome complex reconstituted *in vitro*. A linker histone was site-specifically modified with a single tethered Fe(II) EDTA complex at one end of the globular domain. The redox properties of this complex can be used to generate hydroxyl radicals via Fenton-type

FIGURE 5: Models for the binding site of the globular domain of linker histone within the nucleosome. The core histone octamer is represented by the dark gray cylinder, the DNA by a thick medium gray tube, and the globular domain of linker histone by a light gray sphere. The dyad axis of symmetry passes through the center of each picture, slightly tilted down from perpendicular to the plane of the page.

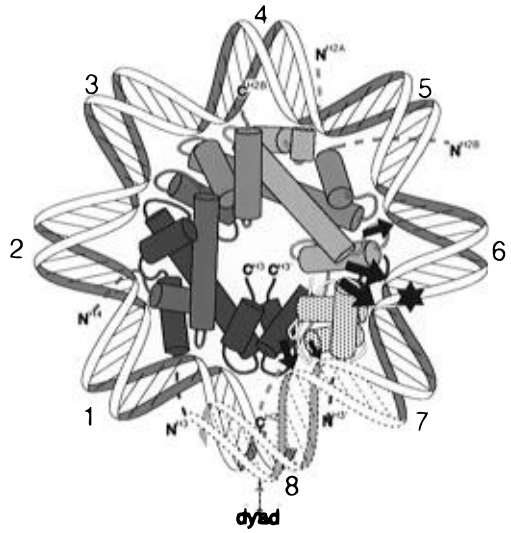

FIGURE 6: Top view of the 5S nucleosome showing the locations of H1°C−Fe(II) EDTA-dependent cleavages. Only protein and DNA in the top half of the nucleosome are shown. Core histone α-helicies (Arents et al., 1991) are represented by columns and other secondary structures as thin tubes as in Pruss et al. (1995). The mobile histone tail domains are indicated by the dashed lines. Histones H3, H4, H2B, and H2A are shaded dark to light gray, respectively. The major sites of H1°C−Fe(II) EDTA-dependent cleavage of 5S nucleosomal DNA are indicated by the black arrows, and the single point of cross-linking detected between GH5 and 5S DNA is indicated by the star. The size of the arrows is qualitatively proportional to the amount of cleavage detected. A predicted location (Pruss et al., 1995) for the three-helical structure of the linker histone globular domain is shown (stippled). Numbers of turns of DNA away from the dyad axis are indicated as in Richmond et al. (1984).

chemistry which will cleave DNA in the vicinity of the metal center (Tullius, 1987). This localized cleavage of the nucleic acid backbone can then be used to precisely map protein−DNA interactions (Ebright et al., 1992; Lavoie et al., 1996; Heilek & Knoller, 1996).

When H1°C−Fe(II) EDTA is reconstituted into the 5S nucleosome, localized cleavage of DNA is found at positions +63 and +73 on the coding strand and at positions +55, +65, and +75 on the noncoding strand of 5S DNA (Figure 6). The character or "envelope" of cleavages at contiguous base positions found at each of these sites suggests damage is due to a freely diffusible radical species emanating from a single point located on the inside of the superhelical gyre of DNA. This point coincides with the predicted location of the linker histone globular domain as shown in Figure 5C (see below). In this model, the globular domain of a single molecule of linker histone interacts asymmetrically with DNA in the nucleosome, occupying a major groove of the DNA, near position +65 in the 5S nucleosome which is about 65 base pairs from where the dyad axis of symmetry passes through the DNA. This position coincides exactly with the location of the cross-link formed between the globular domain of a linker histone and 5S nucleosomal DNA (Hayes et al., 1994). Modeling studies indicate that this site is adjacent to a cleft in the mass of the core histone proteins within the nucleosome into which the linker histone globular domain can fit (Pruss et al., 1995).

The preferential association of the linker histone to one end of the nucleosome generates an asymmetrical particle that may impart a directionality to the folding of the chromatin fiber. This might be propagated by a polar, head to tail arrangement of linker histone molecules along the nucleosomal array within the chromatin fiber (Lennard & Thomas, 1985). Placement within the top superhelical gyre of DNA would also position the globular domain to cause the increase in the superhelical wrapping of DNA that is observed by electron microscopy upon binding of this domain to reconstituted mononucleosome particles (Hamiche et al., 1996). Further, this positioning may then orient the long basic C-terminal tail of linker histone to track along the inside of the continuous solenoid formed by the linker DNA exiting the nucleosome (light gray in Figure 5) (Finch & Klug, 1976).

The results presented in this paper suggest one way that the specific interaction between the linker histone globular domain and the nucleosome core may occur in native chromatin. However, the experiments presented here employ mononucleosome complexes, and it is possible that linker histones adopt a different mode of specific binding when presented with an oligonucleosomal substrate. In addition, different linker histone variants may bind nucleosomes with different modalities. Future experiments with reconstituted oligonucleosomal complexes, different site-specifically modified linker histones, and modified linker histones incorporated into native oligonucleosomes will address these possibilities.

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